The Early Development of the Spleen of Lepidosiren and Protopterus.

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With Plates 15, 16, and 17.

My first words must be those of thanks to Professor Graham Kerr for providing me with every facility for carrying out the research, the results of which are embodied in this short paper. He invited me to work in his laboratory, provided me with material already stained and mounted, and was especially helpful in suggestion and criticism.

Owing to the uncertainty as to the time available for this work, I have done no fresh section-cutting, nor have I restained any of the sections already made. This being so, I found that the earlier stages of Protopterus were more suitable for the present investigation than those of Lepidosiren, and so I will first describe its development in detail, and then contrast and compare that of Lepidosiren, adding further details where I can.

The Spleen of Protopterus.

In the earlier stages than N.T.XXXII.[1] the foregut is short, thick, and solid, its enveloping mesoderm being yolky, and with difficulty distinguishable from the subjacent endoderm.

[1] The stages are designated by the numbers used in Keibel's 'Normentafeln.'

Just about this stage, however, the foregut elongates and narrows, and the mesoderm loses its granules, but remains fairly compact.

On the right-hand side of the foregut, where it overlies the intestine, there is a distinct thickening of its layer of mesoderm, which is particularly vascular. This is where the spleen will arise (fig. 1 and 1a). It is in front of the dorsal rudiment of the pancreas, the only one which has developed at all at this stage, and, except for the posterior lobe of the liver, is entirely posterior to that organ. This posterior lobe is ventro-lateral on the right side of the position of the spleen, but more or less in the mesial plane of the embryo itself, the foregut being to the left of the middle line.

The vascularisation is entirely venous, being part of the gut circulation.

There is nothing to suggest that the endoderm has anything to do with the formation of this thickening, but it is impossible to be dogmatic on the point, because at stages just prior to the one under discussion the two layers seem completely fused, and it is impossible to decide in many instances whether a nucleus belongs to a cell of the endoderm or of the mesoderm. Since, however, all the organs known certainly to be of endodermal origin, e.g. liver, pancreas, or thyroid, arise as heavily-yolked rudiments, therefore the development of the spleen from tissue entirely free from yolk granules and in all other respects resembling the mesenchyme of other parts of the embryo seem to lend support to the view upheld by Laguesse against that by Maurer and Kupffer.

At Stage XXXIII (fig. 2 and 2a), the spleen rudiment is distinctly visible as a flat structure on the right side of the foregut extending as far forward as the point where the latter begins to bend to the mesial plane of the embryo over the lung outgrowth. The anterior ventral portion of the intestine extends for a considerable distance in front of the developing pyloric valve, and so the spleen rudiment is entirely dorsal to it. With regard to the glands of the alimentary canal, it lies behind the origin of the liver and the ventral pancreatic

buds but anterior to the dorsal bud, a position it keeps throughout life.

As yet the cells of the organ are quite undifferentiated from those of the rest of the mesenchyme, but the rudiment is apparent owing to its vascularisation. The vein from the intestine here breaks up into a number of branches which run into the rudiment in a more or less forward and dorsal direction. This is the afferent system. The efferent is formed by a number of small tributaries which unite and enter the liver as the Hepatic Portal Vein. The organ itself contains large sinuses, chiefly peripheral, with which these two systems communicate. They have no visible endothelial lining.

About this time in the development of the embryo the foregut greatly increases in length, and since the spleen lies embedded in its sheath it is to be expected that it would grow in that direction too. Examination of embryos at Stage XXXIV proves this to be the case, its length increasing from about ·2 mm. at Stage XXXIII to about ·5 mm., while its greatest diameter remains at about ·15 mm. Anteriorly it is slightly twisted towards the dorsal side of the foregut, where the latter begins to arch over the lung rudiment: which seems to point to this portion of the foregut being at this stage included in the twisting which occurs chiefly in the intestine. The position of the spleen with regard to the gut appendages is the same as earlier, i.e., the bile-duct opening and the ventral pancreatic buds lie roughly in the same transverse plane as its anterior end, and the dorsal pancreas lies at the posterior end. The venous circulation of this portion of the embryo has undergone no change, except, perhaps, that the peripheral sinuses are better marked, and I have been unable to trace any arterial supply.

There is active cell-division going on throughout the organ, but particularly in the venous spaces, where the erythroblasts are multiplying freely.

At this stage (fig. 3 and 3a) the differentiation between the cells of the spleen and those of the rest of the mesenchyme

commences. The nuclei at the periphery begin to take up a position with their long axes tangential to the organ; the cells containing these will form the capsule. External to these the cells form a rather compact connective tissue, and within them the splenic tissue is but little more differentiated, but its spaces are, of course, venous sinuses.

The alimentary canal between Stages XXXIV and XXXV undergoes a considerable amount of remodelling (I use the word in the same sense as Professor Graham Kerr, 'Quart. Journ. Micr. Sci.,' vol. liv, p. 484). Whereas the first turn of the intestine has up to now been very prominent and has caused the embryo to be rather tadpole-like in shape, at this time there is considerable shrinkage in this region so that there is but little bulging exteriorly (vide Keibel's 'Normentafeln,' vol. x). The result of this is that the relative positions of the organs in connection with the foregut and the anterior portion of the intestine are altered. The foregut itself is rotated so that the spleen lies laterally anteriorly and ventro-laterally posteriorly on its right side: the dorsally placed wall of that part of the intestine projecting in front of the pyloric valve shortens, so that the three pancreatic buds become fused together and the bile-duct opening comes to lie just behind the posterior end of the spleen, a very marked difference from its earlier position: the pancreas extends forwards under the posterior half of the spleen, but gradually retreats further back as development proceeds.

The histology also has advanced, although the tissue is still very condensed. The trabeculæ are developing, as will be seen by examining fig. 4, but the cells, except for their arrangement, are indistinguishable from the rest of the mesenchyme.

By Stage XXXVI, the last one examined, the organ has become very well-marked and obvious in sections, but it never becomes so in dissection, because it remains embedded in the sheath of the foregut. It is just over 1 mm. long, and its greatest diameter, towards its posterior end, is about a third of this.

The first turn of the intestine has shrunk still more, so

that the spleen projects for most of its length, but the posterior end, where it continues to overlap the pancreas, is contained within it.

The histology is as follows: The cells are arranged to form closely-packed trabeculæ, surrounded by blood spaces. These have no endothelial lining, and are the venous sinuses previously mentioned. The peripheral sinuses, which were so well marked in the earlier stages, are now completely broken up by trabeculæ to form the channels of the sponge-work.

The blood supply is rather doubtful. There appears to be a small branch of the cœliac artery acting as an afferent vessel in addition to the branches of the intestinal vein; but, I think, some of the blood from the intestinal goes direct to the liver. The factors of the Hepatic Portal Vein compose, as before, the efferent system.

The Spleen of Lepidosiren.

Turning now to the development of the spleen in Lepidosiren, the differences observable are not of much morphological significance.

It appears at just about the same stage as in Protopterus, but develops rather more quickly, so that by Stage 34, (figs. 5 and 5a) it is aleady about 1 mm. long, and shows the beginnings of a trabecular arrangement among its cells.

Its position, too, differs from that in Protopterus; it is dorsal to the foregut anteriorly, turning over to the right side posteriorly, and lies almost entirely in front of the intestine. This last point of contrast is due to the difference of distribution of yolk in the two species.

I have been unable to discover a branch of the cœliac artery supplying it, but the intestinal vein, besides supplying the spleen, does continue directly to the liver.

Already, however, at Stage 35, a branch of the cœliac artery can be made out going to the spleen, which is as far developed as the latest stage of Protopterus that I have examined (figs. 6 and 6a).

At Stage 36 (figs. 7 and 7a) the organ is clearly defined from the rest of the mesenchyme dorsal to the endodermal wall of the gut. It has sharply-marked boundaries, and is compact, while the neighbouring mesenchyme has developed the alveolar structure typical of ordinary connective-tissue. The capsule-forming cells appear as a single layer of nuclei bounding the organ. These cells have formed a definite connective-tissue sheath over a large portion of the surface by Stage 37, the most advanced stage I have examined.

For the histogenesis of the spleen in Lepidosiren I cannot do better than refer to Dr. Bryce's paper on the "Histology of the Blood of the Larva of Lepidosiren." One point only will I mention. The cellular elements show a marked reduction in size between Stages 35 and 37. This change seems to affect the whole of the mesoderm cells of the foregut, as will be seen by examining figs. 6, 7 and 8. I thought at first that it was due to variation in the amount of contraction which the larvæ had undergone during the preparation of the sections, but that this is not the case is shown by two facts: (1) the blood corpuscles do not show this change, and (2) the effect is observable in all the series examined.

To return to the question of the blood circulation. It is quite clear that this is, to begin with, entirely venous, so that there is a sort of splenic portal system. This is in connection with the veins draining the intestine. The development of these had not been fully worked out in either form, nor have I had the time to do it properly myself, so the following remarks must be accepted with all reserve.

Apparently, in both species the main intestinal vein which drains the intestine breaks up in the spleen. (It will have been noticed that I have referred to the vein which supplies the spleen as the intestinal. There is, of course, no vein ordinarily called by that name: I use it simply as a matter of convenience, because I do not wish to make any definite statement on the point.) The blood from the spleen runs to the

liver viâ the hepatic portal vein. There is, at first, no direct communication between the intestine and the liver, and no arterial supply to the spleen. This latter point seems to be general throughout the phylum, for Dr. Bryce states, in Quain's 'Elements of Anatomy,' vol. i, p. 237, that in the human embryo the artery develops late.

What the chief factor of this intestinal vein is, is uncertain. In Protopterus it appears to be the intra-intestinal, while in Lepidosiren it is the subintestinal. This, however, is most likely only a question of which is most developed at the stage under consideration.

At about Stage XXV in Protopterus, and earlier in Lepidosiren, there is visible a small vein which communicates directly with the liver. This is the Hepatic Portal Vein proper, which, in the adult, becomes a well-marked vessel.

In Lepidosiren at the latest available stage I carefully examined the veins of this region (omitting the smaller tributaries from the intestine). The arrangement is as follows, from before backwards. There are three veins which communicate between the spleen and the hepatic portal vein, and behind the last of these the latter forks; the right branch is confined to the liver, and the left, passing straight through the tissue of the pancreas and turning over the left side of the gut, gradually fades away in the latter's ventral mesenchyme.

I interpret this in this way. The subintestinal vein[1] runs round the left side of the intestine to the dorsal surface, and then gives off a branch running backwards into the right lobe of the liver. It then continues forwards and gives off one branch to the splenic spongework, receives two from it, and then disappears. After reaching the side of the liver the portal vein seems to give off small branches into the liver tissue along the entire length.

[1] Dr. Jane Robertson ('Quart. Journ. Micr. Sci.,' vol. lix, p. 121) describes this vein as the posterior part of the original subintestinal and the proximal part of the left vitelline vein.

W. N. Parker, describing the adult condition in Protopterus, states that the main factor of the hepatic portal is a large mesenteric which runs close to the intra-intestinal artery in the axis of the spiral valve and comes to the surface at the pylorus (this is the intra-intestinal vein of Laguesse). He mentions a subintestinal vein, the connections of which he has not made out, and then says that just anterior to the pylorus the mesenteric can be traced into an anterior and posterior branch, the latter supplying the posterior lobe of the liver behind the gall-bladder. (This is the first branch I have mentioned above.) He continues: "The former receiving a large lieno-gastric vein (the factors of which form a dense meshwork in the spleen) and a pancreatic vein, and then dividing into branches which supply the anterior lobe of the liver." According to him, therefore, there is but one efferent vessel, and the afferent supply is wholly arterial. This is in marked contrast with what obtains in other fish.

(A) T. J. Parker on Mustelus, for instance, describes two large veins connected with the spleen, an anterior and posterior lieno-gastric. The former runs with the lieno-gastric artery, and is most likely efferent; the latter lies between the pyloric division of the stomach and the right lobe (morphological posterior portion) of the spleen and "receives feeders from both."

(B) Laguesse on Acanthias says the hepatic portal is composed of two trunks: the supra-intestinal, running the length of the intestine, and, after passing the hilum of the spleen, receives from that organ the splenic vein; and the subintestinal, which receives blood from the pancreas, at the edge of which it receives the accessory splenic. These two veins correspond to the anterior and posterior lieno-gastric veins of T. J. Parker respectively. He states that in the adult they are anastomosed, and in the embryo it is on this loop that the spleen appears. He also states that there is a double anastomosing arterial supply, one directly from the aorta and one from the cœliac, a condition which, he says, is found in the Trout as well.

Judging, therefore, from these three descriptions, taken with the facts of embryology already known, one comes to the conclusion that the original circulation of the spleen must have been entirely venous, being a portal system between the intestine and the liver. Later it was " shunted " off the main vessel so as to lie on a loop alongside. Later still, the delivery of arterial blood removed the necessity of an afferent venous supply, so that in all forms above the Pisces there are present a single splenic artery and a single splenic vein only. In the class mentioned, however, both the veins persist, and there may be a second artery as well. The direction of the blood-flow in the veins is of some importance, a point on which authors are not very clear. Judging from T. J. Parker's description of Mustelus, it seems as if both the veins are efferent in function. This entails a reversal of the current, in the one serving the right lobe, during development. This is not a serious difficulty (it would be by no means an isolated case), but it makes the efferent system extraordinarily large compared with the afferent, both the veins being so much larger than the artery.

It seems, therefore, as if detailed investigation of the blood-vessels of this part of the body in Lepidosiren and Protopterus would be of much morphological value, and would most likely help to bring into line the various descriptions which have been published for the different fish investigated.

Summary.

(1) The spleen arises in a thickening of the mesenchyme of the foregut, just after that mesenchyme has become free from yolk granules.

(2) It is, at first, a mass of mesenchyme cells, round about which are comparatively large venous sinuses without any endothelial walls; later the cells become arranged to form trabeculæ across these sinuses, which thus get broken up into the channels of a spongework.

(3) The afferent and efferent veins are in very close con-

nection with the veins from the intestine and to the liver respectively. The arterial supply of blood develops from the cœliac artery rather later.

(4) The organ remains throughout ontogeny embedded in the sheath of the foregut, and is therefore inconspicuous.

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EXPLANATION OF PLATES 15, 16, and 17,

Illustrating Mr. G. L. Purser's paper on "The Early Development of the Spleen of Lepidosiren and Protopterus."

All these figures have been drawn with the aid of a Zeiss Abbé drawing apparatus. I have to thank my sister, Miss Dorothy Purser, for making the diagrammatic drawings forming Plate 15.

List of Abbreviations.

ao. Dorsal aorta. *er.* Erythroblasts. *f. g.* Foregut. *g. b.* Gall-bladder. *g. b. d.* Bile-duct. *h. p. v.* Hepatic portal vein. *int.* Intestine. *int. v.* Intestinal vein. *i. v. c.* Inferior vena cava. *k.* Nephridial tubes. *li.* Liver. *lu.* Lung. *pa.* Pancreas. *sp.* Spleen. *sp. a.* Splenic artery. *tr.* Trabeculæ. *v. s.* Venous sinus.

PLATE 15.

Series of diagrammatic figures of transverse sections through the embryos of Protopterus and Lepidosiren, to show the position of the spleen with regard to the neighbouring organs. Their numbers correspond to those of the lithographic figures, which are drawings of the same sections at a higher magnification.

Figs. 1a–4a.—Protopterus. × 20 (circa).

Figs. 5a–8a.—Lepidosiren. × 10 (exc. 5a × 20).

PLATE 16.

Transverse sections through the spleen of Protopterus.

Fig. 1.—N. T. xxxii. × 180.

Fig. 2.—N. T. xxxiii. × 180.

Fig. 3.—N. T. xxxiv. × 220.

Fig. 4.—N. T. xxxv. × 220.

PLATE 17.

Transverse sections through the spleen of Lepidosiren.

Fig. 5.—N.T. 34. × 180.

Fig. 6.—N.T. 35. × 180.

Fig. 7.—N.T. 36. × 180.

Fig. 8.—N.T. 37. × 180.